



Effects of alcohol on insulin-like growth factor I and insulin-like growth factor binding protein 3 in postmenopausal women¹⁻³

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ABSTRACT

Background: Increased circulating insulin-like growth factor I (IGF-I) concentrations, frequently adjusted for IGF binding protein 3 (IGFBP-3), have been associated with increased risk of several types of cancer, including colon, prostate, and breast. Studies have suggested that alcohol may affect IGF-I or IGFBP-3; however, controlled feeding studies to assess alcohol's effects on IGF-I or IGFBP-3 have not been conducted.

Objective: To determine whether chronic, moderate alcohol intake affects serum IGF-I or IGFBP-3 concentrations, we performed a controlled, crossover feeding study.

Design: Fifty-three postmenopausal women were randomly assigned to consume 0 g (control), 15 g (one drink), or 30 g (2 drinks) alcohol daily for 8 wk and were rotated through the other 2 intake levels in random order. All foods and beverages were provided during the intervention. Individuals were monitored and calories adjusted to maintain constant weight, and serum was collected at the end of each diet period.

Results: Compared with the effects of 0 g alcohol/d, IGF-I concentrations were nearly unchanged by 15 g alcohol/d (0.8%; 95% CI: -3.2%, 3.5%) but decreased significantly by 4.9% (95% CI: -8.0%, -1.6%) with 30 g alcohol/d. IGFBP-3 concentrations significantly increased by 3.0% (95% CI: 0.4%, 5.6%) with 15 g alcohol/d but did not increase significantly with 30 g/d (1.8%; 95% CI: -0.9%, 4.5%).

Conclusions: To our knowledge, this is the first published controlled diet study to find that in postmenopausal women, when weight is kept constant, alcohol consumption reduces the amount of serum IGF-I potentially available for receptor binding. These findings suggest that the effect of alcohol intake should be considered in studies of IGF-I, IGFBP-3, and cancer in postmenopausal women. *Am J Clin Nutr* 2005;81:503-7.

KEY WORDS Alcohol, insulin-like growth factor I, insulin-like growth factor binding protein 3, postmenopausal women, controlled feeding study

INTRODUCTION

Insulin-like growth factor I (IGF-I) is an endocrine hormone that also has autocrine and paracrine roles in many tissues (1-3). The hormone is associated with cell proliferation and anti-apoptosis, and its actions are mediated through the IGF-I receptor. Postnatal circulating concentrations of IGF-I increase

throughout childhood, spike during puberty when the hormone plays a critical role in long bone growth, and decrease slowly with age thereafter. Although interindividual variation is considerable, blood concentrations of IGF-I are relatively constant on a day-to-day basis for a given individual during a given period in life. There are 6 major IGF binding proteins, and >90% of circulating IGF-I is bound to IGF binding proteins (IGFBPs), mainly IGFBP-3. IGFBP-bound IGF-I is unavailable for receptor binding and activation (2).

The IGF system has been shown to be involved in the regulation of postprandial glucose concentrations, renal function, and bone mineral density in healthy individuals (3). Recent epidemiologic evidence, however, indicates that increased serum concentrations of IGF-I, either alone or expressed relative to concentrations of IGFBP-3, are associated with an increased risk of several cancers, including prostate (4), colorectal (5-13), lung (14-17), and breast (18-30) cancer. Of the multiple studies that have investigated the association between breast cancer and IGF, several studies found a positive association between IGF-I or IGF-I adjusted for IGFBP-3 and risk of breast cancer in analyses of premenopausal women (19, 20, 24, 25, 28), whereas 2 found a positive association in analyses of postmenopausal women (25, 26).

Because of its association with cancer risk, many recent studies have investigated whether certain modifiable dietary factors are associated with increases in serum IGF-I concentrations and could therefore affect associations between IGF and cancer. Several studies have indicated, for example, that dietary energy intake is associated with IGF-I (2, 31). Fasting appears to result in decreased (32, 33) and overfeeding in increased (34) IGF-I,

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although the exact dose-response relation between food intake and blood IGF-I concentrations is not yet well understood. Among the other dietary factors that may affect IGF-I, alcohol is of interest because it is commonly consumed and is independently associated with breast cancer risk (35). A few cross-sectional epidemiologic studies have investigated the association between moderate alcohol consumption and IGF-I or IGFBP-3 concentrations in women, but the results of these studies have been mixed. One study found a positive association between alcohol consumption and IGF-I in women aged ≥ 50 y (36), and 2 found no association among women aged 30–84 y (37) or 43–69 y (38). Of the 2 cross-sectional studies that investigated the association between alcohol intake and IGFBP-3 concentrations in women, one found a positive association (38) and one found no association (37). Because these studies were all cross-sectional in design, many study population differences, including age, race, alcohol intake, and other dietary factors, were not controlled for and could account for the discrepancies in the study findings.

To better understand the association between alcohol and circulating concentrations of IGF-I and IGFBP-3, we assessed the effect of moderate alcohol intake on IGF-I and IGFBP-3 in a controlled, crossover feeding study in postmenopausal women. The women were given a balanced, controlled diet with 0, 15, or 30 g alcohol/d for 8 wk and were rotated randomly through the other 2 alcohol levels for 8 wk each. We used this type of study design so that alterations seen in serum proteins could be attributed to alcohol intake alone. Understanding whether alcohol intake affects circulating concentrations of IGF-I and IGFBP-3 is important given that these proteins have been associated with various cancers and have been shown to affect cell proliferation and survival, two very important factors in carcinogenesis.

SUBJECTS AND METHODS

Participants and study design

The postmenopausal Women's Alcohol Study was conducted from 1998 through 1999 at the US Department of Agriculture's Beltsville Human Nutrition Research Center and has been previously described in detail (39–41). Briefly, participants were healthy postmenopausal women aged ≥ 50 y who were not using estrogen replacement therapy or prescription medications that could interfere with the study. They were nonsmokers within 90–140% of the ideal body weight for their height. Each woman was screened to confirm that she had no major health problems. Exclusion criteria included being an abstainer from alcohol, having a history of alcohol abuse, or having parents with a history of alcoholism. This study was approved by the institutional review boards of the National Cancer Institute and the Johns Hopkins University Bloomberg School of Public Health. Participants signed informed consent forms before entering the study and were compensated for their participation. A total of 63 women were enrolled and randomly assigned in the study, and 53 completed the entire course.

The dietary intervention used a 3-period crossover design. Women were randomly assigned to consume 1 of 3 alcohol levels (0, 15, or 30 g/d) for 8 wk and were rotated through the other 2 categories in random order. Each woman consumed only food and beverages provided by the study during the 3 diet periods, and energy levels were adjusted to keep body weight constant. A

2- to 5-wk washout period was interposed between each diet and alcohol period, and during that time women consumed no alcohol but had no other food restrictions.

All foods and beverages were prepared and supplied by the Beltsville Human Nutrition Research Center's Human Studies Facility, and all subjects ate the same foods. Diets provided 15% of calories from protein, 35% from fat, and 50% from carbohydrates or carbohydrates plus alcohol. Alcohol (Everclear; Daniel Sherman Corp, St Louis) was supplied in orange juice, and energy from alcohol was compensated for in the 0- or 15-g alcohol diets by supplemental carbohydrates from soft drinks or Polycose (Ross Products Division, Abbott Laboratories, Columbus, OH). Participants were instructed to consume their study beverages at home, 1–2 h before bedtime and after all activities requiring manual dexterity, such as driving, were complete. Compliance was encouraged by administration of a daily questionnaire that asked the women whether they had consumed their study beverage the night before and by periodic random spiking of each participants' study beverage with riboflavin followed by collection and testing of morning spot urine samples.

During the last (ie, eighth) week of each study period, blood samples were collected on 3 separate days, and equal volumes of each sample were pooled for analyses. All samples were collected after the women had fasted overnight.

Laboratory methods

IGF-I and IGFBP-3 concentrations in pooled serum were individually measured by enzyme-linked immunosorbent assay (both assays from Diagnostic Systems Laboratories Inc, Webster, TX). All samples from a single individual were run in the same batch. Two aliquots from each pooled sample were assayed in duplicate. If the CV of these 4 assays was $>10\%$, the samples were reassayed in the same manner. All values were then averaged to generate a final concentration. Blinded control aliquots from a single source were assayed on each plate, and CVs from these samples were determined to be 6.2% for IGF-I ($n = 20$) and 8.9% for IGFBP-3 ($n = 20$).

Statistical analysis

IGF-I and IGFBP-3 concentrations were transformed to the natural log before statistical analyses so that treatment effects could be evaluated as relative changes and error terms would be approximately normally distributed. Linear mixed models (42) with a single random intercept reflecting a subject effect were used to estimate and test for changes in IGF-I, IGFBP-3, and the ratio of IGF-I to IGFBP-3 at 15 and 30 g alcohol/d relative to the placebo group. Models were fit treating the alcohol groups as 2 indicator variables. A likelihood ratio test (chi-square with 2 df was used as a global test for treatment effect). We evaluated carryover effects by testing for order effects in the models, but found none for IGF-I, IGFBP-3, or the ratio of IGF-I to IGFBP-3. Models containing a term for body mass index (BMI) were fit to adjust for the potential confounding effects of BMI; estimates and tests were nearly identical for models with and without an adjustment for BMI. Effect modification by age, BMI, and height was examined by testing for an interaction between the variable of interest and dose groups. All reported P values are based on two-sided tests.

TABLE 1

Geometric mean of serum insulin-like growth factor I (IGF-I), IGF binding protein 3 (IGFBP-3), and the ratio of IGF-I to IGFBP-3 in the 53 study participants when consuming 0, 15, or 30 g alcohol/d and percentage change from no alcohol to 15 or 30 g alcohol/d¹

Alcohol level	IGF-I ²	IGFBP-3 ³	IGF-I/IGFBP-3 ⁴
0 g alcohol/d			
\bar{x} (ng/mL)	129.8 (121.2, 138.9)	2260.6 (2150.4, 2376.5)	0.057 (0.055, 0.060)
15 g alcohol/d			
\bar{x} (ng/mL)	130.9 (121.8, 140.6)	2328.6 (2214.0, 2449.1)	0.056 (0.053, 0.060)
Percentage change (%) ⁵	0.8 (−3.2, 3.5)	3.0 (0.4, 5.6)	−2.1 (1.3, −5.4)
30 g alcohol/d			
\bar{x} (ng/mL)	123.5 (114.7, 133.1)	2299.9 (2178.7, 2427.9)	0.54 (0.051, 0.057)
Percentage change (%) ⁵	−4.9 (−1.6, −8.0)	1.8 (−0.9, 4.5)	−6.5 (−3.2, −9.6)

¹ 95% CI in parentheses.

² $P = 0.002$ for global test of 0 versus 15 versus 30 g/d.

³ $P = 0.08$ for global test of 0 versus 15 versus 30 g/d.

⁴ $P < 0.001$ for global test of 0 versus 15 versus 30 g/d.

⁵ Estimates are from linear mixed models, including a random intercept reflecting a subject effect and alcohol groups as indicator variables. Percentage changes and CIs were nearly identical when we adjusted for BMI.

RESULTS

The study participants' baseline characteristics were determined before consumption of the controlled diets and were described previously (39). Briefly, the women's average age was 59.7 y (range: 49.2–78.8 y), and their average height and weight were 1.64 m (range: 1.52–1.80 m) and 74.4 kg (range: 42.1–117.4 kg), respectively. Seventy-four percent of study subjects were white, 23% were African American, and 4% were Asian. Eighty-five percent were parous, and 83% had a natural menopause as opposed to a hysterectomy.

Serum IGF-I and IGFBP-3 concentrations and the ratio of IGF-I to IGFBP-3 in the no alcohol period are reported in **Table 1**, along with the percentage change with alcohol consumption. As compared with no alcohol, serum IGF-I concentrations in women were nearly unchanged with 15 g alcohol/d but decreased by 4.9% with 30 g/d ($P = 0.002$ for global test of 0 versus 15 versus 30 g/d). IGFBP-3 concentrations increased by 3.0% and 1.8% for 15 and 30 g alcohol/d relative to 0 g/d, respectively ($P = 0.08$ for global test of 0 versus 15 versus 30 g/d). The ratio of IGF-I to IGFBP-3 decreased by 2.1% and 6.5% for 15 and 30 g alcohol/d relative to 0 g/d ($P < 0.001$). We found no evidence that age, height, or BMI modified the effects of alcohol on either IGF-I or IGFBP-3 or the ratio of IGF-I to IGFBP-3.

DISCUSSION

Our results indicate that moderate alcohol consumption over an 8-wk period affects serum concentrations of both IGF-I and its main binding protein, IGFBP-3. Thirty grams of alcohol per day decreased IGF-I concentrations, although 15 g/d had no significant effect. IGFBP-3 concentrations, on the other hand, were increased by 15 g alcohol/d but were only marginally increased by 30 g/d. These results indicate that, when diet and weight are kept constant, moderate, chronic alcohol consumption potentially reduces the amount of serum IGF-I available for receptor binding.

To our knowledge, this is the first controlled diet study to investigate the relation between alcohol intake and serum concentrations of IGF-I and IGFBP-3 in postmenopausal women.

Our IGF-I findings are in contrast with several previous cross-sectional studies in women (36–38); however, they are consistent with a recent cross-sectional study in middle-aged Japanese men (43) and an acute alcohol intake study in 23–29-y-old male and female medical students (44). The latter study showed that serum IGF-I concentrations declined 7 h after 3 doses of 0.45 g alcohol/kg body wt was given. A single dose from that study is approximately equal to the 30-g alcohol dose for the average-weight woman in the present study. Our IGFBP-3 findings are also consistent with a cross-sectional study that examined the association between alcohol intake and serum IGFBP-3 concentrations in women (38). Whereas the cross-sectional studies assessed alcohol consumption by questionnaires, which are subject to misclassification and confounding from unmeasured factors, the design of our study, an intervention with a crossover design using controlled diets, virtually ensures that the alterations in IGF-I and IGFBP-3 we report were due to alcohol and not to some other dietary component.

We recently completed the analysis of data from a similar, controlled, crossover feeding study in premenopausal women (45). In that study, only 2 levels of alcohol, 0 or 30 g/d, were assessed, and we observed that with 30 g alcohol/d, IGF-I concentrations were significantly decreased by 9.5% compared with no alcohol. This change was almost twice that seen in the current study, but it was in the same direction as in postmenopausal women. IGF-I concentrations tend to decline with age (1, 3); thus, the difference in the magnitude of the IGF-I decrease seen with 30 g alcohol/d may be due to the fact that the same amount of alcohol has a greater effect when IGF-I concentrations are higher. Alternatively, some other factor present only in premenopausal women could be affecting IGF-I concentrations in the presence of alcohol. Regarding IGFBP-3, concentrations were unchanged in the premenopausal women's study by 30 g alcohol/d (45). This is somewhat in contrast with the present study findings, but 15 g alcohol/d, which was the dose at which we saw a significant effect on IGFBP-3 in postmenopausal women, was not investigated in the premenopausal study. It is therefore unclear whether the significant IGFBP-3 increase seen at 15 g alcohol/d is generalizable.



If an increase in serum IGF-I or a decrease in IGFBP-3 had been observed in response to alcohol exposure, it would have supported the hypothesis that alcohol intake contributes to breast cancer through IGF-I. However, we observed an effect opposite of our expectation. One possible explanation for our results could be that alcohol intake by study subjects affected growth hormone concentrations, which are known to regulate liver IGF-I production (46). Previously, Ekman et al (47) showed that acute low-dose alcohol intake in young adults (ie, 0.5 or 1.0 g/kg), significantly reduced the growth hormone surge normally seen at 0100. Although these authors did not evaluate IGF-I concentrations, it seems likely that the daily intake of alcohol protocol used in our study could have reduced the growth hormone peak in our study subjects and consequently decreased liver production of IGF-I. A possible side effect of these changes would be long-term reductions in bone density (48); thus, it would be interesting to assess measures of bone mass in the current study sample. Our finding that 15 g alcohol/d increased IGFBP-3 is interesting given that growth hormone also positively regulates this protein (49). However, we saw a significant decrease in IGF-I only at the higher dose and a significant increase in IGFBP-3 only at the lower dose of alcohol. The daily dose of alcohol therefore appears to have a complex influence on the IGF axis, in a manner that is independent of alcohol's effects on breast cancer risk.

A recent meta-analysis determined that alcohol intake increases breast cancer risk by 7.1% for each 10 g of alcohol consumed per day (35). The sample of women studied here has been evaluated for other effects of alcohol intake (39–41, 50), and the results have shown the complicated, sometimes beneficial, sometimes detrimental effects of alcohol ingestion. For example, Dorgan et al (39) showed that alcohol increased serum concentrations of sulfated estrone and dihydroepiandrosterone, which presumably increase available estrogen concentrations and, therefore, breast cancer risk. Furthermore, Roth et al (50) recently found that serum leptin concentrations increased with increasing alcohol intake and proposed that this increase could contribute to carcinogenesis. In contrast, Davies et al showed that alcohol reduced insulin and enhanced insulin sensitivity (40), whereas Baer et al (41) found that alcohol intake improved lipid profiles in these women. Added to the current results of reduced IGF-I and increased IGFBP-3, findings from studies of these women have shown that alcohol has positive effects in postmenopausal women that could reduce their heart disease risk (40, 41) and has mixed effects on cancer risk and bone density by both increasing circulating sulfated estrogen (39) and leptin (50, 51) and decreasing serum IGF-I concentrations. Further studies will be needed to understand whether and how each individual's cancer risk may be affected by alcohol's overall effects. To date, increased concentrations of the serum IGF-I proteins have been positively associated with colon, lung, and breast cancer, diseases that are important in older women. Given our current findings and that alcohol intake could vary by disease status, it will be important to assess the effect of alcohol intake on the relation between IGF and cancer in future studies of postmenopausal women.

Although our study was modest in size, the highly controlled nature and crossover design allowed us to identify the effects of alcohol, when weight and diet were kept constant, on IGF-I and IGFBP-3 concentrations. Possible weaknesses in the study include the potential lack of generalizability to other free-living populations; however, as mentioned above, we did see a decrease

in IGF-I concentrations in a similar study in premenopausal women (45). We also tested only 2 doses of alcohol in the relatively low to moderate range, thus providing a limited picture of the dose-response relation studied. At the levels of alcohol ingestion studied here, we cannot ascertain what would happen with greater alcohol intake; however, it is known that chronic heavy drinking causes liver damage and decreases concentrations of IGF-I, because the liver is the main source of circulating IGF-I (52). Finally, only 8 wk of intake was tested; thus, we remain uninformed about what would happen over shorter or longer periods of time. However, if growth hormone is responsible in part for the effects we observed, the studies discussed above indicate that changes in growth hormone have almost immediate effects on IGF-I concentrations (47).

In summary, we showed for the first time that under controlled, isocaloric dietary conditions, moderate, chronic alcohol consumption in postmenopausal women increases serum IGFBP-3 concentrations or reduces serum IGF-I concentrations and therefore reduces the amount of IGF-I potentially able to bind to its receptor and subsequently initiate its biological signaling cascade. Our findings suggest that because alcohol and IGF-I concentrations are both associated with increased breast cancer risk and because alcohol decreases IGF-I, alcohol intake could affect the association between IGF-I and breast cancer. Future studies of IGF-I, IGFBP-3, and cancer in postmenopausal women should carefully consider the effects of alcohol intake on the study findings.



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